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C. Gelfand  
PATENT 2/11/93

Atty. Docket No. 8636

GROUP 180

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : David H. Gelfand et al.  
SERIAL NO. : 07/873,897 GROUP ART UNIT: 1808  
FILED : April 24, 1992 EXAMINER: D. Haff  
DOCKET NO. : 8636  
TITLE : PURIFIED THERMOSTABLE ENZYME

DECLARATION UNDER 37 C.F.R. §1.131

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

We, David H. Gelfand, Randall K. Saiki, and Susanne Stoffel do hereby declare as follows:

1. Co-inventor David H. Gelfand received a Bachelor of Arts degree in Biology from Brandeis University and a Doctorate degree in Biology from the University of California. Co-inventor Gelfand was employed by Cetus Corporation from December, 1976, to December, 1991, and attained the position of Vice President, Scientific Affairs.

2. Co-inventor Randy K. Saiki received a Bachelor of Arts in Chemistry degree and a Bachelor of Science in Biology degree from the University of Washington. Co-inventor Saiki was employed by Cetus Corporation from October, 1979, to December, 1991, and attained the position of Scientist.

3. Co-inventor Susanne Stoffel received a diploma from the University of Zurich. Co-inventor Stoffel was employed by Cetus Corporation from October, 1978, to December, 1991, and attained the position of Research Associate II.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of patents and Trademarks, Washington, D.C. 20231, on this 15 day of January, 1993  
By David H. Gelfand

4. Prior to 1990, we held other positions at Cetus Corporation, which are shown in the curriculum vitae attached hereto as Exhibit A. In 1991 the rights to PCR technology were purchased by Hoffmann-La Roche Inc. from Cetus Corporation. We were among the Cetus Corporation employees who were offered a position at Roche Molecular Systems, Inc. Roche Molecular Systems Inc. is a wholly owned subsidiary of Hoffmann-La Roche, Inc., the present owner of the subject patent application.

5. We, David H. Gelfand, Randy K. Saiki, and Susanne Stoffel, are co-inventors of the claimed subject matter of the above-captioned application. We are familiar with the Office Actions mailed September 4, 1990; May 3, 1991; and July 15, 1992, in which the Examiner rejected claims of this application over the MBR product information sheet of June 8, 1987. Prior to May 1, 1987, we reduced the claimed invention to practice in the United States. Evidence of our reduction to practice is shown by the Exhibits and the discussion below.

6. Evidence of the reduction to practice and conception is set forth in Exhibits B, C, and D. Exhibit B comprises copies of notebook pages 110-113 and 115-118 from laboratory notebook No. 2233 assigned to co-inventor Saiki. The dates on the pages, all prior to May 1, 1987, have been blanked out. Item 7, below, is a discussion of the relevant portions of Exhibit B (a copy of which is annexed hereto).

7. At pages 110-113 and 115-118 co-inventor Saiki recorded initial titration experiments for determining the optimum amount of Taq polymerase "Lot 2" in a PCR assay. "Lot 2" was purified by co-inventors Gelfand and Stoffel according to the protocol described in the above-captioned patent application. The results of that experiment, summarized at page 118, demonstrated that 1/8 µl enzyme, equivalent to 1.5 units, was optimal in the PCR.

8. Exhibit C comprises copies of notebook pages 81, 82, 90, 92, and 101-105 from laboratory notebook No. 2369 assigned to co-inventor Saiki. The dates on the pages, all prior to May 1, 1987, have been blanked out. Items 9-18, below, are a discussion of the relevant portions of Exhibit C (a copy of which is annexed hereto).

9. At pages 81 and 82, co-inventor Saiki recorded initial titration experiments for determining the optimum amount of Taq polymerase "Lot 3" in a PCR assay. "Lot 3" was purified by co-inventors Gelfand and Stoffel according to the protocol described in the above-captioned patent application. The results of that experiment demonstrated that in the PCR conditions described, PCR product was visible using as little as 1.25 units of Taq per reaction.

10. At pages 90 and 92 co-inventor Saiki described an experiment to compare titrations of "Lot 2" and "Lot 3" Taq polymerase. At the time of the experiment described at page 90, "Lot 2" enzyme had been in use for over four months since the titration described at Exhibit B. "Lot 3" enzyme had been in use for one week. The results of the experiment, summarized at page 91, show that "Lot 2" enzyme had not lost any activity during four months of storage.

11. At page 101 co-inventor Saiki recorded that "Lot 2" enzyme storage buffer contained non-ionic detergent and did not contain gelatin.

12. At page 101 co-inventor Saiki recorded that the storage buffer for "Lot 3" contained gelatin, but not non-ionic detergent.

13. Co-inventor Saiki, at page 101, recorded the observation that over time Taq polymerase, "Lot 3," was losing activity. In two to three weeks the activity had diminished by at least four fold. At that time, co-inventors Gelfand and Stoffel were using non-ionic detergent during the purification of Taq polymerase as well as during assays for stimulating enzyme activity. At page 101, co-inventor Saiki recorded the observation that experiments were needed to determine the effect of non-ionic detergent in the storage buffer, and in the PCR reaction, on enzyme activity.

14. The experiment initiated at page 101 and continued through page 103 is an analysis of serially diluted Taq polymerase "Lot 3" in PCR mixtures with and without 0.5% non-ionic detergent.

15. At page 103 co-inventor Saiki recorded the observation that "Detergents definitely had some effect." Further, the entry notes that enzyme activity, even with detergent in the PCR mix, was not high as when "Lot 3" was initially titrated, approximately three weeks earlier.

16. At page 103 co-inventor Saiki described that investigating the effects of adding non-ionic detergent to the storage buffer, rather than the PCR mix for stabilizing enzyme activity would be the next experiment.

17. At page 104 co-inventor Saiki described an experiment for determining the effect of adding non-ionic detergent directly to the enzyme storage buffer. In that experiment "Lot 3" Taq polymerase was serially diluted from storage buffer with and without non-ionic detergent.

18. At page 105 co-inventor Saiki recorded the results of the experiment described at page 104. The experiment demonstrated that (1) adding non-ionic detergent to the storage buffer was superior for stabilizing enzyme activity than adding non-ionic detergent to the PCR; (2) the full amount of enzyme activity originally titrated was restored by the addition of non-ionic detergent to the storage buffer; and (3) without detergent the deterioration of enzyme activity had continued with further storage.

19. Further evidence of the reduction to practice of the claimed invention is provided by Exhibit D. Exhibit D comprises copies of notebook pages 57-59 from laboratory notebook No. 2610, assigned to co-inventor Saiki. The dates on these pages, all prior to June 8, 1987, have been blanked out. The following is a discussion of the relevant portions of Exhibit D (a copy of which is annexed hereto).

20. The experiment described at pages 57-59 of notebook No. 2610 was conducted by co-inventor Saiki in response to the observation that Taq polymerase purchased from New England Biolabs (NEB) was losing activity upon storage. At page 57 the fact was recorded that, as a result of work done and described above and in Exhibit C, as well as additional experiments by co-inventors Stoffel and Gelfand, we had recognized the problem of diminished activity upon storage and we had subsequently solved the problem by adding non-ionic detergents to the enzyme storage buffer.

21. At page 59 of notebook No. 2610, the results of the experiment for restoring enzyme activity to the NEB product are provided. The experiment demonstrates and the notebook states that the "addition of detergent to the storage buffer fully restores activity of the enzyme."

22. Having seen these results and having discussed the data amongst ourselves, we realized that the addition of non-ionic detergent to a preparation of purified thermostable DNA polymerase would provide a storage stable enzyme composition.

We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

David H. Gelfand  
David H. Gelfand

1/6/93  
Date

Randall K. Saiki  
Randall K. Saiki

06 Jan 93  
Date

Susanne Stoffel  
Susanne Stoffel

Jan 6<sup>th</sup> 93  
Date